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Ontogenesis of Circadian Pituitary–Adrenal Periodicity in Rats Affected by Neonatal Treatment wth ACTH[1] (37845)

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It is well-established that cyclic gonadotropin release in the female rat can be abolished by administering sex steroids during a "critical period" of early postnatal development (1). A similar phenomenon has recently been reported for the pituitary–adrenocortical system in rats (2). Krieger (2) observed suppression of the 24-hr adrenocortical rhythm in 30-day-old rats injected with corticosteroids between postnatal days 2–4. Corticosteroids administered during postnatal days 12–14 failed to alter the rhythm, suggesting that the earlier treatment interfered with the development of a neuroendocrine regulatory mechanism during a critical period. It is uncertain, however, whether suppression of the adrenocortical rhythm in 30-day-old rats indicates that the rhythm will remain suppressed through adulthood or merely that its appearance has been delayed since: (a) the 24-hr adrenocortical rhythm in rats has been reported to mature as early as the third (3) and as late as the fifth postnatal week (4); and (b) administration of corticosteroids to infant rats delays the maturation of a variety of physiological processes, including morphological and functional development of the brain (5). The results of the present experiment demonstrate that elevations of adrenocorticotropic hormone (ACTH)-mediated corticosteroid levels and/or ACTH itself, during specific periods of postnatal development, modify the circadian pituitary–adrenocortical rhythm in adult rats.

Methods. Litters of Long–Evans rats were housed under conditions of controlled temperature on a 14-hr light:10-hr dark cycle (lights on 4 AM–6 PM). On the day of birth, all pups were cross-fostered, and 4 males and 4 females were randomly assigned to the dams in the experimental groups. These groups consisted of litters of noninjected controls and litters injected subcutaneously with either ACTH (0.4 IU/g body wt; H. P. Acthar Gel, Armour Pharmaceutical Co., Chicago) or an equal volume of the gel vehicle (16% gelatin [BACTO Gelatin, DIFCO Certified, DIFCO Labs., Detroit], 0.5% phenol, 0.1% cysteine) for 3 consecutive days beginning in the morning of postnatal day 2, 7, 12, 17, 22, or 27. On the first day of injection, all rats were toe- and ear-marked for subsequent identification. All rats were weaned and group-housed by sex on day 30.

At 120 days of age, morning (AM) basal samples were obtained between 8 and 11 AM, the time of the normal diurnal trough in plasma steroid levels in our laboratory. Blood samples (0.5 ml) were withdrawn by cardiac puncture within 1 min of removing the rat from its home cage. Afternoon (PM) basal samples were obtained in the

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same manner between 5 and 6 PM, the time of the normal diurnal peak in plasma steroid levels under our conditions. One week intervened between AM and PM sampling. The blood was immediately centrifuged and the plasma removed and frozen for subsequent analysis of corticosterone by a microfluorometric method (6).

The entire experiment was replicated on two occasions separated by a six-months' interval. Since there was no difference in the distribution of the means in the two replications, the data from both experiments were pooled for final statistical analysis and presentation. After determination of significant effects by analysis of variance (Treatment × Period of Injection), Student's *t* test was used to compare the ACTH- and gel-treated groups with each other and with the noninjected control groups.

Results and Discussion. The dosage of ACTH used in these experiments was sufficient to produce adrenocortical activation at 3–6 hr following single injections into neonatal rats at all ages tested (unpublished observations). On postnatal day 30, all ACTH-injected rats, except those receiving ACTH on days 17–19, weighed less ($P < .05$) than noninjected controls. Male rats injected with gel vehicle on days 12–14, 22–24, and 27–29 and females injected on days 22–24 also weighed less ($P < .01$) than noninjected controls on day 30; however, by day 50, these differences in body weights had disappeared. Adrenal weights (mg/100 g body wt) obtained in male rats upon autopsy in adulthood indicated that males injected with ACTH on days 12–14 and 27–29 had smaller ($P < .05$) adrenals and those receiving gel on days 2–4 had larger ($P < .05$) adrenals than noninjected controls; adrenal weights of all other groups were comparable to control weights.

Analysis of variance (Treatment × Period of Injection) indicated overall significant differences among days of injection for AM steroid values for males ($F(5,151) = 6.3$, $P < .01$) and females ($F(5,113) = 7.25$, $P < .01$), PM steroid values for males ($F(5,151) = 3.24$, $P < .01$) and females ($F(5,115) = 2.28$, $P = .05$) and the percentage change from AM to PM values for males ($F(5,151) = 3.91$, $P < .01$) and females ($F(5,113) = 3.58$, $P < .01$).

Inspection of the steroid data presented in Table I reveals that ACTH- (males) or gel-treatment (females) on days 17–19 produced AM levels which were elevated ($P < .05$) above those of nontreated control rats. ACTH- (females) or gel-treatment (males) on days 22–24 or either treatment on days 27–29 in females resulted in adult AM steroid levels significantly lower than those of nontreated controls. These effects on AM levels were not specifically related to either treatment since there were no significant differences between steroid levels in groups receiving ACTH or gel during the same postnatal period.

Afternoon steroid levels were lower ($P < .05$) than those of non-treated animals in male rats receiving ACTH on days 7–9, 17–19, or 22–24 or gel on days 27–29 (Table I). ACTH on days 22–24 produced similar effects in female rats. In the case of the day 7–9 and 17–19 treatments, their suppressive effects on PM steroid levels in males appeared to be specifically related to ACTH: PM levels in males treated with gel at corresponding ages were similar to those of nontreated rats and higher ($P < .05$) than those of the ACTH-treated animals.

It is apparent from the steroid data in Table I that all PM values were significantly higher than their corresponding AM values except in one group, i.e., females receiving gel on days 17–19. Thus, only the latter group of animals showed a virtual loss of its circadian rhythm. In order to determine the extent of suppression of the rhythm, the percentage change from AM to PM values was calculated for each animal; the resulting means and standard errors for each group are presented in Fig. 1. The smallest percentage changes, i.e., the greatest suppression of the circadian rhythm, were observed in both adult males and females injected with ACTH neonatally on days 7–9 or 17–19 and in females injected with gel on days 17–19 (Fig. 1). The per-

TABLE I. Effects of Neonatal Treatment with ACTH or Gel Vehicle on the Circadian Variation in Plasma Corticosterone Levels Determined at the Trough (AM) and Peak (PM) of the Normal Daily Cycle in Adult Rats.

Treatment age (days)	Male			Female		
	No. of rats	AM	PM	No. of rats	AM	PM
		Corticosterone, μg/100 ml plasma (Mean ± SEM)			Corticosterone, μg/100 ml plasma (Mean ± SEM)	
None	19	8.5 ± 0.6	20.8 ± 1.5	15	17.1 ± 1.9	36.2 ± 2.9
ACTH						
2–4	9	9.3 ± 0.9	19.5 ± 1.5	4	18.4 ± 3.9	35.3 ± 6.5
7–9	12	10.2 ± 0.9	15.5 ± 0.7[a,c]	9	19.9 ± 2.0	30.6 ± 1.8
12–14	14	6.9 ± 0.8	21.3 ± 1.8	12	15.9 ± 2.1	37.3 ± 2.5
17–19	12	11.1 ± 0.9[a]	15.9 ± 1.4[a,c]	9	18.7 ± 1.8	32.2 ± 1.0
22–24	18	7.5 ± 0.8	15.2 ± 1.3[a]	16	10.5 ± 1.4[b]	27.5 ± 1.9[a]
27–29	21	7.9 ± 0.5	16.4 ± 1.4	11	11.3 ± 1.4[a]	30.3 ± 1.9
Gel						
2–4	15	6.8 ± 0.8	18.3 ± 1.2	13	17.4 ± 1.8	35.8 ± 1.7
7–9	11	9.3 ± 1.1	19.1 ± 1.7	15	14.8 ± 2.1	37.6 ± 2.2
12–14	11	8.4 ± 1.1	17.3 ± 1.3	9	16.0 ± 1.7	32.9 ± 2.5
17–19	9	10.2 ± 1.2	20.3 ± 1.2	11	24.2 ± 2.7[a]	31.1 ± 4.0
22–24	13	5.8 ± 0.6[b]	19.1 ± 1.7	9	12.6 ± 2.5	29.2 ± 3.2
27–29	18	7.1 ± 0.6	14.5 ± 0.8[b]	7	10.4 ± 1.6[a]	32.5 ± 1.8

[a] $P < .05$ compared to untreated animals.
[b] $P < .01$ compared to untreated animals.
[c] $P < .05$ compared to corresponding gel-treated animals.

centage changes at these two periods in the males were lower ($P < .01$) than those of nontreated animals; however, the percentages for the females failed to be significantly different from those of nontreated animals. It may be that the large variability in basal steroid levels in the females masked the age-dependent effects observable in the males. This variability may have been due to the sampling of females at various stages of the estrous cycle (7).

Based on statistical comparison of the percentage changes in ACTH- and gel-treated groups of corresponding ages, it can be concluded that the reduced AM–PM percentages were specifically related to treatment with ACTH on days 7–9 ($P < 0.1$) and 17–19 ($P < .05$) in males and 7–9 in females ($P < .05$); gel treatment at these ages did not produce similar effects. The failure to find a significant difference between the two treatments on days 17–19 in females reflects the apparent suppressive effects of both treatments on the AM–PM percentage change at this period in females. The finding of an effect of gel treatment during days 17–19 in females, as discussed above, but not in males, suggests differential interactions between adrenocortical and gonadal steroids in males and females during development. The highly significant difference between the percentage changes in males treated on days 22–24 is more a reflection of an augmented change in the gel-treated group than a reduction in the ACTH-treated animals.

In conclusion, these data indicate that elevated AM steroid levels and/or reduced PM levels led to a suppressed circadian rhythm, as represented by reduced AM–PM percentage changes, only in adult rats which had been treated neonatally on days 7–9 or 17–19. Thus, two neonatal periods have been identified during which alterations in ACTH-mediated corticosteroid levels and/or ACTH itself modify the subsequent development of the circadian pituitary–adrenocortical rhythm. This is another demonstration of the important role of the hormonal milieu in the maturation of phy-

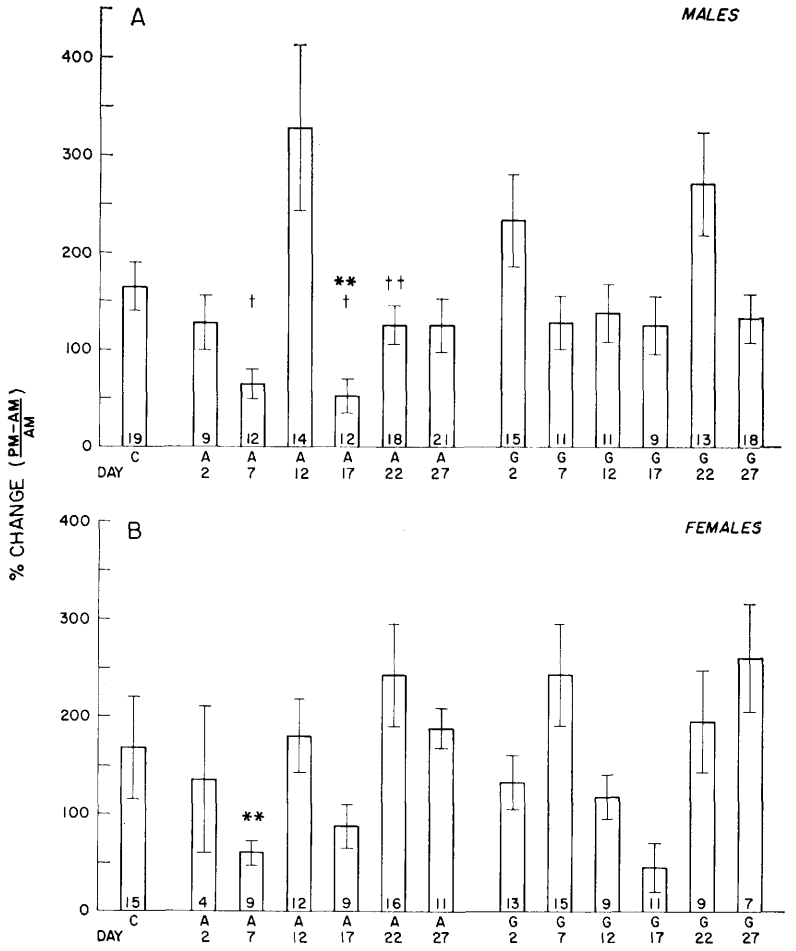

FIG. 1. Effects of treatment with ACTH or gel vehicle at various ages postnatally on the circadian variation in plasma corticosterone levels in adult male (A) and female (B) rats represented as the percentage change from AM (trough) to PM (peak) levels. Abbreviations and symbols: (C) noninjected control rats; (A) ACTH; (G) gel vehicle; day, number indicates age on first of 3 consecutive injection days; numbers inside columns, the number of rats in each experimental group; vertical bars, ± 1 SE; single dagger, $P < .01$ compared to noninjected controls; double daggers, $P < .01$ compared to corresponding gel-treated animals; double asterisks, $P < .05$ compared to corresponding gel-treated animals.

siological processes (1, 5). The mechanism of hormonal action in influencing the development of the central neural substrates for these processes remains unknown. The first critical period (days 7–9) corresponds to the time when the hypothalamo–hypophyseal–portal system is developing and the period of maximum effectiveness of steroid hormones on hypothalamic differentiation (8, 9). The second period (days 17–19) occurs at a time when hypothalamic differentiation is complete and neural cell proliferation and differentiation is taking place in the forebrain (9). Hypothalamic and forebrain regions have both been implicated in the central control of the circadian pituitary–adrenocortical rhythm in adult rats (11, 12). Thus, it is conceivable that the altered hormonal milieu may have exerted a permanent effect on the circadian rhythm by directly influencing the development and maturation of these two central regions. The present data, collected at only two times during the 24-hr cycle, do not reveal the

extent of the disruptive effect on the rhythm; nevertheless, the elevation of AM (trough) values and depression of PM (peak) values are consistent with the effects produced by hypothalamic deafferentation (12) or fornix transection (13).

Summary. Neonatal treatment with ACTH on days 7–9 and 17–19 suppressed the circadian corticosteroid rhythm in adult rats. Treatment with gel vehicle at these ages, except in females on days 17–19, or with ACTH or gel vehicle at other times neonatally did not produce similar effects. Thus, brief exposure to high circulating levels of ACTH and/or corticosteroids during two critical neonatal periods affects the normal development of mechanisms underlying cyclic pituitary–adrenal function in the rat.

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